BODY SIZE AS A FACTOR IN THE METAMORPHOSIS OF TADPOLES

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Introduction

The rôle of body size in the activities of organisms has been studied only in a comparative way. Its effectiveness can be demonstrated experimentally, however, within a single species in relation to a number of physiological functions. One of these functions in Amphibia is metamorphosis.

When many tadpoles were reared in one aquarium, as is described in the preceding paper, they were retarded markedly in growth as compared with isolated individuals. When the time came for the isolated individuals to metamorphose, certain of the partially crowded individuals were also able to metamorphose. But the body weights of the latter were much less than those of the isolated individuals. Other tadpoles crowded more densely did not metamorphose at this time, but were able to metamorphose at later times. To analyse these relationships, data were obtained under controlled conditions upon the changes of body weight and the ages at which the morphological changes of metamorphosis occurred.

In the preceding paper size was regarded as a result conditioned *inter alia* by crowding. In the present paper size is to be considered as a condition, the result produced being *inter alia* metamorphosis.

Two species, *Rana sylvatica* and *Rana pipiens*, were used in the observations, and the ages at metamorphosis were recorded for about 190 individuals that had been reared under known conditions. In selected instances the body weights of single individuals were measured; in other instances groups of individuals were followed with respect to body weight through metamorphosis. That crowding resulted in delay in tadpole metamorphosis was reported by Yung (1885), but no data on body weight were obtained by him. The present experiments were recently summarized in abstract (Adolph, 1930).

Weight Changes During Metamorphosis

The progressive changes of body weight in tadpoles crowded to varying extents are shown in Fig. 1. Some time after the increase of body weight of the tadpole has fallen below the initial "logarithmic" rate, the percentage increment is greatly reduced, and then increase ceases. Finally body weight is lost rapidly for about two weeks, at the end of which time metamorphosis is visibly complete.

The tadpole has ceased to grow before most of the morphological changes of metamorphosis are apparent. The first changes are the budding of the hindlegs; no others are observable ordinarily until the tadpole has begun to lose weight.

After metamorphosis is complete, the body weight of the frog, if not fed, is almost constant for many days. There is a slight gradual loss due to the fact that body tissue is being used as the source of metabolic materials. Three or four weeks (at 19° C.) after the maximum weight of the tadpole is reached, the final weight of the metamorphosed frog is attained. On the average 60 per cent of the body weight is lost in this four-week period.

The forelegs usually burst forth from under the skin when about one-third of the metamorphic loss of weight has occurred. This event is the most convenient morphological event to identify, and in the present study it has been used as the criterion of metamorphosis. But when thus referred to the changes of body weight it is found that the appearance of the forelegs varies considerably in time of occurrence. If a single criterion of metamorphosis is required, the best one would seem to be the point at which the body weight is halfway between the maximum weight of the tadpole and the weight of the frog three or four weeks (or an equivalent age at any other temperature) thereafter; for the change in body weight represents an average of all the changes that are occurring in the body.

The tremendous loss of weight during metamorphosis does not represent a 60 per cent reduction of all chemical constituents of the body. While it is well known that catabolism of nitrogen and other substances is increased at this time, the losses through catabolism do not nearly correspond to the losses of weight. The percentage of solids is known to increase markedly (Schaper, 1902), and this change alone accounts for a considerable portion of the loss of body weight.

Body Weight and Metamorphosis

At 19° C. the maximum weight attained before metamorphosis in *Rana sylvatica,* brood *Q,* was 1230 mg. and in *Rana pipiens,* brood *U,* was 3425 mg.

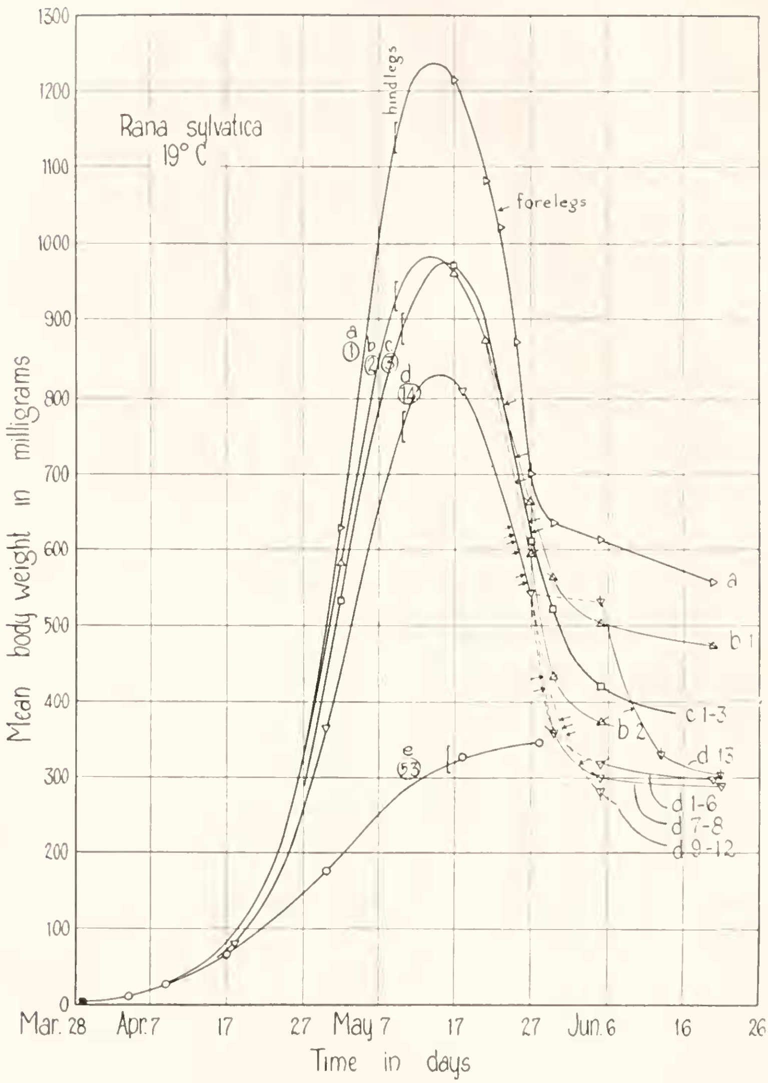

FIG. 1. Growth in weight of five cultures of *Rana sylvatica*, brood *Q*, at 19° C. The numbers of individuals contained in the cultures are indicated in circles; each culture was in 1000 cc. of tap water, having a surface of 550 sq. cm. and a depth of 1.8 cm., that was changed once a week. The first appearance of hindlegs in the culture is indicated by a bracket, and the appearance of forelegs, which was taken as the sign of metamorphosis, is indicated for each individual by an arrow. The body weights after metamorphosis were determined for smaller groups of individuals which were numbered in the order of metamorphosis. The subsequent history of culture *e* is indicated in Fig. 4.

When the uncrowded tadpoles of a brood metamorphosed, the slightly crowded individuals also metamorphosed, and each of the events marking this transformation occurred upon almost exactly the same day for all individuals. But, as Fig. 1 shows, the body weights that had been attained upon the day when the decrease of weight began were diverse. With a density of fourteen individuals per liter the mean weight was only two-thirds of the weight where the density was one per liter.

During metamorphosis the same relative differences of size were maintained, so that the resulting frogs were of diverse sizes. There was, however, a slight tendency for the smaller tadpoles to lose a larger percentage of their body weights in the transformation process. Hence the percentage diversity of sizes was somewhat greater among the complete frogs than among the tadpoles.

The great contrast in the sizes of frogs is illustrated by the frequency curves of maximum weight represented in Fig. 2. The sizes

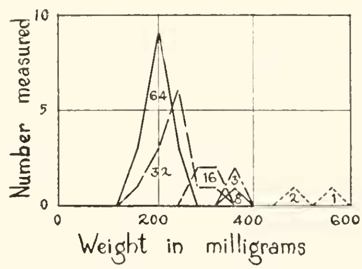

FIG. 2. Frequency distribution of the final weights after metamorphosis in all cultures of *Rana sylvatica*, brood *Q*, at 19° C. The numbers indicate the initial densities of the tadpole populations in individuals per 1000 cc.

are thus shown to depend primarily upon the amount of growth that was previously allowed by the density of the tadpole population (or other limiting factor).

In addition to knowing the sizes attained by individuals that metamorphosed, it is important to know the sizes of tadpoles that did not metamorphose. Is there any sharp limit of body weight that determines whether or not metamorphosis shall occur? The maximum weights attained by the largest tadpoles not metamorphosing and the other tadpoles metamorphosing are given for one brood of *Rana pipiens* in Table I. So far as data are available, they indicate that body weight constitutes a decisive quantitative factor in metamorphosis. At 19° C. the upper limit of size that did not allow metamorphosis within 300 days after fertilization was 2200 mg. for brood *U, Rana pipiens* (individuals *ta* and *va*), and within 150 days was about 550 mg. for brood *Q, Rana* *sylvatica;* in the latter brood the tadpoles that did not eventually metamorphose were very few.

Age at Metamorphosis

At 19° C. uncrowded individuals of *Rana sylvatica,* brood *Q,* acquired forelegs at the age of 54 days. In *Rana pipiens,* brood *U,* the corresponding stage was attained at 117 days. The tadpoles that were slightly crowded were able to metamorphose at the same time as uncrowded ones. Hence within certain limits the body size had little influence upon the time of onset of metamorphosis. But tadpoles that were densely crowded did not metamorphose at the same age as uncrowded ones. This is apparent in the two densest populations of Fig. 1.

Table I

Ages and weights during metamorphosis of individuals of Rana pipiens, brood U, at 19° C.

Designation	Age at Appearance of Forelegs	Body Weights on the 105th Day	Maximum Body Weight	Body Weight at Appearance of Forelegs	Final Body Weight After Metamorphosis
	days	*mg.*	*mg.*	*mg.*	*mg.*
ca	117	2735	2735	1400	—
j	117	3425	3425	2450	—
la	118	2730	2770	2060	—
ua	119	—	—	1510	1300
qaa	120	2880	2890	1860	1360
ub	148	—	2750	—	1110
raa	152	—	2860	—	—
oa	225	—	—	—	—
ta	—	1910	—	—	—
va	—	2000	—	—	—
rab	343	—	—	—	—
qab	344	1585	—	—	—
ma	457	1930	—	—	—

If the frequency of various ages at which metamorphosis occurs is plotted, as in Fig. 3, the contrast is great. The most densely crowded individuals not only never metamorphosed at so young an age as uncrowded ones, but various individuals metamorphosed at highly diverse times.

The diversity of ages at which metamorphosis occurred is illustrated in detail in Fig. 4. Over a period of more than two months transformations frequently occurred in the particular culture illustrated. Of course, the transformations cannot be said to have occurred at random, for in each case it was usually the largest tadpole that began to metamorphose next.

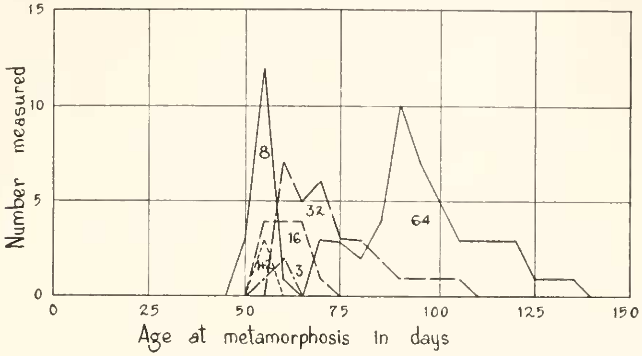

FIG. 3. Frequency distribution of the times (ages) of appearance of forelegs in all cultures of *Rana sylvatica*, brood *Q*, at 19° C. The numbers indicate the initial densities of the tadpole populations.

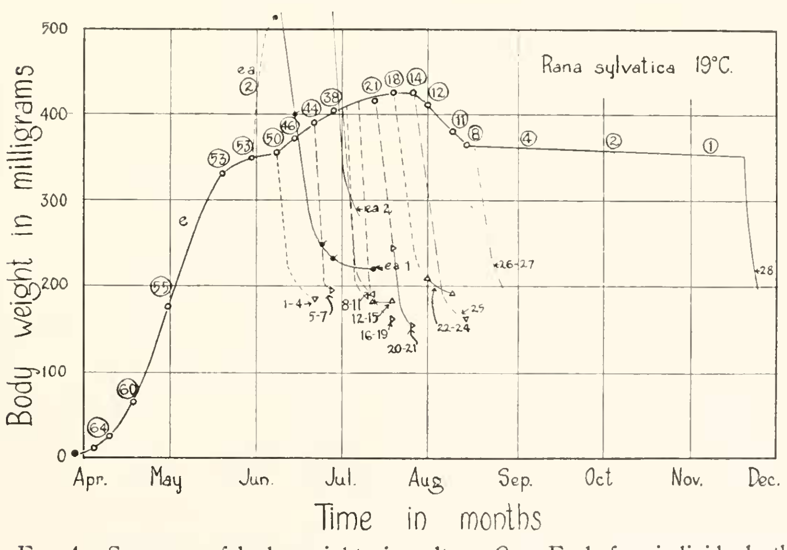

FIG. 4. Sequence of body weights in culture *Qe*. Each few individuals that metamorphosed were weighed after the forelegs had appeared, the individuals being numbered consecutively as they metamorphosed. The total numbers of individuals in the culture are indicated in circles. Two average individuals *ea* were isolated into 1000 cc. before any had metamorphosed; these were able to grow considerably before they transformed.

In the brood shown in Fig. 4, the last individual that had survived came to metamorphose 237 days after its growth started. Its age was then 440 per cent of the age when the uncrowded individuals of the same brood metamorphosed, which may be referred to as "par." In *Rana pipiens* the last survivor metamorphosed at the age of 457 days (Table I), which was 390 per cent of par. While metamorphoses are frequent at ages near par, they become less frequent per unit of the population exposed to metamorphosis as age increases. This is due not to the death rate among the retarded tadpoles, but to the fact that the condition which must be met before metamorphosis can occur, which is body size itself, becomes slower in rate of attainment.

Body Weight and Age

The interaction of the two factors of metamorphosis, namely, size and age, may now be evaluated. It was found, as shown in Table I, that individuals that were just on the verge of attaining the size necessary for metamorphosis were still able to metamorphose after a delay of some weeks or months, even though they made little or no further gain in weight. The charts of body weight indicate that metamorphosis to the extent of stopping growth in weight might be said actually to have occurred at par age, but the morphological changes of metamorphosis did not proceed. Evidently, within certain limits, a deficiency in body size can be compensated by an increase of age.

The way to compare the rôles of the size factor and the age factor in metamorphosis is to plot the two together. This is done in Fig. 5 for the one brood on which most data are available. Since the known body weights are more numerous after the completion of metamorphosis than at the beginning of metamorphosis, the final weight of the frog is used as the measure of body size. The same sort of curve results, however, whether maximum weight of the tadpole or weight on the day that forelegs are acquired be used in place of final weight of the frog.

The best curve drawn empirically through the points of Fig. 5 is a rectangular hyperbola. If W is the body weight in milligrams after the completion of metamorphosis, A is the age at which the forelegs broke through in days after fertilization of the egg, and c, d, and e are constants, the relationship $(A - e)(W - d) = c$ represents the graph. The constants d and e represent the asymptotes of the hyperbola. The conclusion may be drawn that no possible increase of size would allow metamorphosis to occur before e days of age, and neoteny would last indefinitely if sufficient body size to result in a frog of weight d were not attained.

For the brood Q at 19° C., living on the diet of *Spirogyra, Vaucheria,* and spinach used, the minimum age e is 51 days, the minimum body weight d is 160 mg., and the constant c is 1200 day-milligrams.

The curve as drawn in Fig. 5 represents these values. Under the conditions in which brood *Q* was reared, the influence of other factors upon the initiation of metamorphosis was evidently small. Body weight and age were the effective factors in conditioning the onset of metamorphosis.

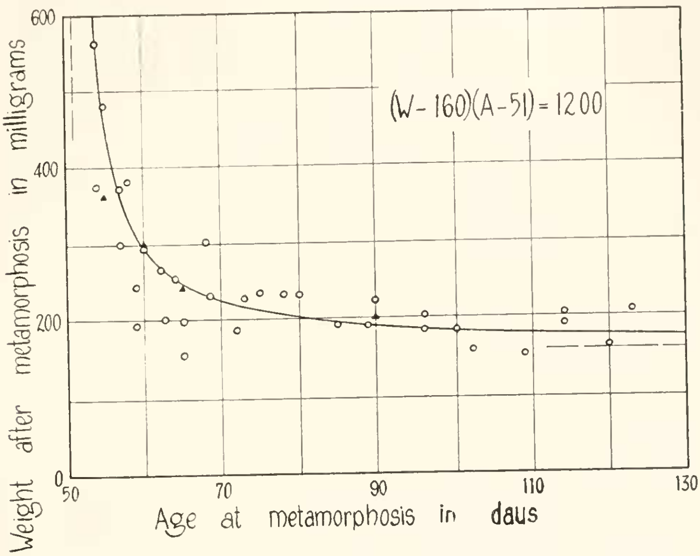

Fig. 5. Correlation between the ages at which the forelegs appeared and the weights of the frogs after metamorphosis was complete, in all individuals of *Rana sylvatica,* brood *Q,* at 19° C. The curve drawn through the points is represented by the formula for a hyperbola, the dashed lines being the asymptotes. The four solid points represent the modes for various density groups read off from Figs. 2 and 3.

Comment

The factors that have been held to be responsible for initiating metamorphosis in Amphibia may be roughly classified as: Age, size, previous history, food, oxygen supply, temperature, hormonal relations, and heredity. The rôles of age and size have been evaluated above by using observations in which the other factors were held largely constant. If under previous history be included rate of growth and crowding, then it has been shown that these are of importance chiefly because they influence size.

Types of food have not been varied in these experiments; and it may be that all the observations reported by others in which the food was varied really influenced metamorphosis either through size or through endocrine mechanisms. Starvation was reported by Barfurth (1887) to initiate metamorphosis in frogs. If his data are analyzed, however, it is found that by no objective test of significance were his starved individuals different from his fed individuals. Powers (1903) concluded from careful observations that sudden starvation precipitated metamorphosis in *Amblystoma*. Several attempts were made during the present experiments to bring on metamorphosis by denying food to tadpoles that had almost attained the minimum size required for metamorphosis. But none metamorphosed without further feeding.

High oxygen tensions were stated by Huxley (1925) to inhibit the metamorphosis of frogs. Extirpation of the lung rudiments by Helff (1931) had no significant effect upon the time of metamorphosis. The necessity of rising to the surface for air, the amount of gill surface, and the contact with air are said by Powers (1903) to be of no consequence in the metamorphosis of *Amblystoma*.

The temperature was held constant in the present experiments. Uhlenhuth (1919, 1921) reported that when grown at low temperatures, certain urodeles not merely took longer to attain metamorphosis, but grew to a larger size before metamorphosis.

The influence of heredity has never been studied in frogs apart from environmental factors. That broods differ within the same species is possibly indicated by the varying reports of size at metamorphosis. Thus, in *Rana pipiens* Kuntz (1924) reported that the maximum size of the tadpoles was 6.8 grams, while Helff (1926) reported that the maximum size was 1.4 grams. In both cases, nevertheless, 57 per cent of the body weight was lost before metamorphosis was complete.

The evaluation of size as a factor in determining the onset of metamorphosis does not imply that size is an independent variable. When all the factors are quantitatively known, it will probably be found that most of them are both effects and causes. It may be that one or another chemical or physical condition will appear to set aside the usual complex of conditions. It is already known that thyroid feeding will render the size factor unnecessary for metamorphosis; very small and young tadpoles thereby attain adult properties. But the rate of an endocrine activity is coördinately correlated with many other factors, and it would be almost anomalous if it ultimately proved true that a single limiting factor ordinarily controlled the onset of metamorphosis.

For anyone who desires to visualize a causal concatenation of factors, a schema to which the author does not subscribe, a possible mechanism by which size and metamorphosis are correlated may be pictured as follows. It is well known that metamorphosis is often controlled by the activity of the thyroid gland. This gland is ordinarily thrown into sufficient activity to induce metamorphosis only in the presence of anterior pituitary tissue from metamorphosing tadpoles (Smith, 1923;

Uhlenhuth, 1928; Ingram, 1929). But the anterior pituitary also often controls the rate of growth, and hence the body size (Smith, 1918; Allen, 1920). The anterior pituitary must ordinarily attain its ability to set off metamorphosis through its developmental age, but in addition cannot actually set off metamorphosis unless it and other organs have attained a certain size through growth. The failure to attain this minimal size or activity, either absolute or relative, may be due not merely to insufficient nutriment, but equally well to any other influences retarding growth.

Many important physiological reasons may be postulated as to why a tadpole much smaller than the usual should not metamorphose. It is doubtful whether the relative objective importance of any of these reasons could be evaluated. In nature all sizes from a few milligrams to many grams, and all ages from a few days to several years are found to be sufficient for metamorphosis in one amphibian species or another. Almost no physiological block to metamorphosis cannot, it may be supposed, be overcome in the course of evolution. Only the situation as found in particular species can be described as a fit part of the pattern of existence.

Summary

1. Tadpoles of *Rana sylvatica* and *R. pipiens* if sufficiently retarded in growth by crowding did not metamorphose at the same ages as uncrowded ones. They were able to metamorphose at their small sizes at later times. Those only slightly retarded were able to metamorphose at the usual time, becoming small frogs.

2. Within certain limits a deficiency of body weight is compensated by a surplus of age, and a correlation of the two factors has been established. Through retardation of growth in size the larval stage can be greatly prolonged. Body size is therefore a tangible quantitative factor in the complex of conditions which regulate the onset of metamorphosis.

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